Study Details

Test No. 2016368

Product Name

Cas-No:	EC-No:	Chemical Name:
1897392-68-5		4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel-

Product code		
Product Name		
Test code		
Purity	95,3	(if 0,0 then see remarks)
Batch No.	Ho154262-MM+0.1% Vit. E.	
Study code	1783503	
Institute Name	Envigo CRS GmbH (former HARLAN)	
Description	Bacterial Reverse Mutation test (Ames), OECD 471, EU B.13/14	
Final Report date	27.01.2017	
Results	not genotoxic	
Reliability	Rel 1	
GLP	YES	
Remark	AMES test using strains TA98, TA100, TA1535, TA1537 and E. coli WP2 uvrA up to 5000 µg/plate, cytotoxic effects occurred with and without S9, precipitation at 1000-5000µg/plate in 1.experiment and 2500-5000µg/plate in the 2.experiment Rel. 1: according to OECD 471 (1997), EU B.13/14 (Commission Regulation 440/2008/EC, 2008) and GLP	

ENVIGO

Report

Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay

Envigo Study Number:	1783503
Envigo Reference Number:	TV29SX
Sponsor Name:	
Version ID:	Final – first original of three
Issue Date:	27 January 2017
Study Director:	Dr. Steffi Chang
Testing Facility:	Envigo CRS GmbH In den Leppsteinswiesen 19 64380 Rossdorf Germany

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ANNEXES

COMPLIANCE WITH GOOD LABORATORY PRACTICE

Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel-: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

- Chemikaliengesetz" (Chemicals Act) of the Federal Republic of Germany, "Anhang 1" (Annex 1) in its currently valid version
- OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17
- EC Commission Directive 2004/10/EC

These principles are compatible with Good Laboratory Practice regulations specified by regulatory authorities throughout the European Community, the United States (EPA and FDA), and Japan (MHLW, MAFF and METI), and other countries that are signatories to the OECD Mutual Acceptance of Data Agreement.

S. Chang

Dr. Steffi Chang
Study Director
Envigo CRS GmbH

27 January 2017
Date

QUALITY ASSURANCE STATEMENT

Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel-: *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay

Study based activities at the Test Facility Envigo CRS GmbH were audited and inspected. The details of these audits and inspections are given below.

Type of Inspection	Date(s) of Inspection	Date Reporting to Study Director, Test Facility Management
Study Plan Verification	12 July 2016	12 July 2016
Study Plan Amendment 1	23 December 2016	23 December 2016
Process – based Test item preparation	13 July 2016	13 July 2016
Report Audit	16 September 2016	16 September 2016
Report Audit 2	06 October 2016	06 October 2016

General facilities and activities where this study was conducted were inspected on an annual basis and results are reported to the relevant responsible person and Management.

S. Ebert

27 January 2017

Date

Sabine Ebert

Quality Assurance Auditor
Envigo CRS GmbH

1 SUMMARY

This study was performed to investigate the potential of Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel- to induce gene mutations according to the plate incorporation test (experiment I and Ia) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and the *Escherichia coli* strain WP2 *uvrA*. Due to a calculation error in experiment I the applied concentrations were not in compliance with the OECD 471 guideline. Therefore the experiment was repeated and reported as experiment Ia. Since the repeat was performed after experiment II and the dose selection for experiment II was based on the results of experiment I all collected data are reported.
The assay was performed in three independent experiments all with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.
The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 2.7; 9.0; 29.6; 89.6; 298.2; 895.6; 2239; and 4478 µg/plate

Pre-Experiment/Experiment Ia: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II:

TA 100: 3; 10; 33; 100; 333; 1000; and 2500 µg/plate
All remaining strains: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

The test item precipitated in the overlay agar in the test tubes at a concentration of 895.6 µg/plate and above in experiment I; from 1000 to 5000 µg/plate in the experiment Ia and at 2500 and 5000 µg/plate in experiment II. No precipitation of the test item in the overlay agar on the incubated agar plates was observed.

The plates incubated with the test item showed reduced background growth with and without S9 mix in all strains used.

Toxic effects, evident as a reduction in the number of revertants (below the indication factor of 0.5), occurred in all strains used.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel- at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.
Therefore, Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel- is considered to be non-mutagenic in this *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay.

2 INTRODUCTION AND PURPOSE

The experiments were performed to assess the potential of the test item to induce gene mutations by means of two independent *Salmonella typhimurium* and *Escherichia coli* reverse mutation assays. Experiment I was performed as a plate incorporation assay. Due to a calculation error the applied concentrations were not according to the OECD guideline. The experiment was repeated and reported as experiment Ia. Experiment Ia was performed after experiment II. Since the dose selection was based on the results of Experiment I the collected data are reported. Experiment I and Ia were both negative and a second experiment carried out as a pre incubation assay was also performed.

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency at which an agent abolishes or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to grow in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.
The *Salmonella typhimurium* histidine (his) and the *Escherichia coli* tryptophan (trp) reversion system measures $his^- \rightarrow his^+$ and $trp^- \rightarrow trp^+$ reversions, respectively. The *Salmonella typhimurium* and *Escherichia coli* strains are constructed to differentiate between base pair (TA 1535, TA 100, and WP2 *uvrA*) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation or the pre-incubation method the bacteria are exposed to the test item with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect at least seven dose levels with adequately spaced concentrations were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens were tested in parallel to the test item.

2.1 Study Details

Sponsor

Study Monitor

Deputy Study Director

2.2 Study Schedule

Experimental start date 13 July 2016

Experimental completion date 28 September 2016

2.3 Regulatory Testing Guidelines

This study was designed to be compatible with the procedures indicated by the following internationally accepted guidelines and recommendations:

- Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471: Bacterial Reverse Mutation Test, adopted July 21, 1997
- Commission Regulation (EC) No. 440/2008 B13/14, dated May 30, 2008

3 MATERIALS AND METHODS

3.1 Test Item and Supporting Information

Information as provided by the Sponsor.

Identification:	Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel-
Batch:	Ho 154 262 MM + 0.1% Vit.E.
CAS No.:	1897392-68-5 and 132130- 08-6
Purity:	95.3%
Appearance:	Colorless, clear, liquid
Expiry Date:	December 2017
Storage Conditions:	At room temperature, protected from light*
Stability in solvent:	Not indicated by the sponsor

* Only valid for storage conditions, not for test performance.

The dose selection was adjusted to purity.

3.2 Study Controls

3.2.1 Negative Controls

Concurrent untreated and solvent controls were performed.

3.2.2 Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Purity:	at least 99 %
Dissolved in:	deionised water
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Purity:	> 99.9 %
Dissolved in:	DMSO (purity >99 %)
Concentration:	10 µg/plate in strain TA 98, 50 µg/plate in strain TA 1537

Strain:	WP2 *uvrA*
Name:	methyl methane sulfonate, MMS
Purity:	> 99.0 %
Dissolved in:	deionised water
Concentration:	2.0 µL/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, WP2 *uvrA*
Name:	2-aminoanthracene, 2-AA
Purity:	97.5 %
Dissolved in:	DMSO (purity >99 %)
Concentration:	2.5 µg/plate (10.0 µg/plate in WP2 *uvrA*)

The stability of the positive control substances in solution is unknown but a mutagenic response in the expected range are sufficient evidence of biological stability.

3.3 Test Item Preparation

On the day of the experiment, the test item Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel- was dissolved in Ethanol (purity > 99 %). The solvent was chosen because of its solubility properties and its relative nontoxicity to the bacteria.

All formulations were prepared freshly before treatment and used within two hours of preparation. The formulation was assumed to be stable for this period unless specified otherwise by the Sponsor.

3.4 Test System

3.4.1 Characterisation of the *Salmonella typhimurium* Strains and *Escherichia coli* Strain

The histidine dependent strains are derived from *Salmonella typhimurium* strain LT2 through mutations in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker.

Strain WP2 and its derivatives all carry the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent (Trp^+) mutants (revertants) can arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility can occur in several different ways so that the system seems capable of detecting all types of mutagen which substitute one base for another. Additionally, the *uvrA* derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.

When summarized the mutations of the TA strains and the *E. coli* strain, used in this study can be described as follows:

Strains	Genotype	Type of mutations indicated
Salmonella typhimurium		
TA 1537	*his* C 3076; *rfa⁻; uvrB⁻*	frame shift mutations
TA 98	*his* D 3052; *rfa⁻; uvrB⁻*; R-factor	" "
TA 1535	*his* G 46; *rfa⁻; uvrB⁻*	base-pair substitutions
TA 100	*his* G 46; *rfa⁻; uvrB⁻*; R-factor	" "
Escherichia coli		
WP2 *uvrA*	*trp⁻; uvrA⁻*	base-pair substitutions and others

Regular checking of the properties of the *Salmonella typhimurium* and *Escherichia coli* strains regarding the membrane permeability, ampicillin resistance; UV sensitivity, and amino acid requirement as well as normal spontaneous mutation rates is performed in Envigo CRS GmbH according to B. Ames *et al.* and D. Maron and B. Ames. In this way it is ensured that the experimental conditions set down by Ames are fulfilled.

The bacterial strains TA 1535, TA 1537, TA 98, TA 100, and WP2 *uvrA* were obtained from Trinova Biochem GmbH (35394 Gießen, Germany).

3.4.2 Storage

The strain cultures are stored as stock cultures in ampoules with nutrient broth + 5 % DMSO in liquid nitrogen.

3.4.3 Precultures

The thawed bacterial suspension was transferred into 250 mL Erlenmeyer flasks containing 50 mL nutrient medium. A solution of 50 µL ampicillin (25 µg/mL) was added to the strains TA 98 and TA 100. This nutrient medium contains per litre:

8 g Nutrient Broth
5 g NaCl

The bacterial cultures were incubated in a shaking water bath for 4 hours at 37° C. The optical density of the bacteria was determined by absorption measurement and the obtained values indicated that the bacteria were harvested at the late exponential or early stationary phase (10^8-10^9 cells/mL).

3.4.4 Selective Agar

Plates with selective agar (without histidine/tryptophan) were used.

3.4.5 Overlay Agar

The overlay agar contains per litre:

for *Salmonella typhimurium*:	for *Escherichia coli*:
7.0 g Agar Agar	7.0 g Agar Agar
6.0 g NaCl	6.0 g NaCl
10.5 mg L-Histidine×HCl×H_2O	10.2 mg Tryptophan
12.2 mg Biotin	

Sterilisations were performed at 121 °C in an autoclave.

3.5 Mammalian Microsomal Fraction S9 Mix

Due to the limited capacity for metabolic activation of potential mutagens in *in vitro* methods an exogenous metabolic activation system is necessary.
Phenobarbital/β-naphthoflavone induced rat liver S9 were used as the metabolic activation system. The S9 was prepared and stored according to the currently valid version of the SOP for rat liver S9 preparation. Each batch of S9 was routinely tested for its capability to activate the known mutagens benzo[a]pyrene and 2-aminoanthracene in the Ames test.
The protein concentration of the S9 preparation was 26.0 mg/mL (Lot. No.: 210116E) in the experiment Ia and 26.2 mg/mL (Lot. No.: 210116K) in the experiment I and II.

3.5.1 S9 Mix

An appropriate quantity of S9 supernatant is thawed and mixed with S9 cofactor solution, to result in a final concentration of approx. 10 % v/v in the S9 mix. Cofactors are added to the S9 mix to reach the following concentrations in the S9 mix:

8 mM	$MgCl_2$
33 mM	KCl
5 mM	glucose-6-phosphate
4 mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
During the experiment, the S9 mix is stored in an ice bath. The S9 mix preparation is performed according to Ames et al.

3.5.2 S9 Mix Substitution Buffer

The S9 mix substitution buffer contains per litre:

700 mL 100 mM sodium-ortho-phosphate-buffer pH 7.4
300 mL KCl solution 0.15 M

During the experiment, the S9 mix substitution buffer is stored in an ice bath

3.6 Experimental Design and Study Conduct

3.6.1 Pre-Experiment for Toxicity

To evaluate the toxicity of the test item a pre-experiment was performed with all strains used. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I and Ia below (plate incorporation test).

Toxicity of the test item can be evident as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.
Due to a calculation error in pre-experiment I the following concentrations were applied:
2.7; 9.0; 29.6; 89.6; 298.2; 895.6; 2239; and 4478 µg/plate
Since the top dose was not according to the OECD guideline the experiment was repeated and reported as pre-experiment Ia. Here, test concentrations were according to guideline.
In both experiments the following criteria were met:
Evaluable plates (>0 colonies) at five concentrations or more in all strains used.

3.6.2 Dose Selection

In the experiment I the concentration range of the test item was 2.7 – 4478 µg/plate. Based on the results of experiment I 5000 µg/plate were chosen as maximal concentration in experiment II.
The concentration range included two logarithmic decades. The following concentrations were tested in experiment II:

TA 100:	3; 10; 33; 100; 333; 1000; and 2500 µg/plate
All remaining strains:	10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

3.6.3 Experimental Performance

For each strain and dose level, including the controls, three plates were used.
The following materials were mixed in a test tube and poured onto the selective agar plates:

Experiment I and Ia (Plate Incorporation)

100 µL Test solution at each dose level (solvent or reference mutagen solution (positive control)),

500 µL S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),

100 µL Bacteria suspension (cf. test system, pre-culture of the strains),

2000 µL Overlay agar

Experiment II (Pre-Incubation)

In the pre-incubation assay 50 µL test solution (solvent or reference mutagen solution (positive control)), 500 µL S9 mix / S9 mix substitution buffer and 100 µL bacterial suspension were mixed in a test tube and incubated at 37 °C for 60 minutes. After pre-incubation 2.0 mL overlay agar (45 °C) was added to each tube. The mixture was poured on minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37 °C in the dark.
In parallel to each test a sterile control of the test item was performed and documented in the raw data. Therefore, 100 µL of the stock solution, 500 µl S9 mix / S9 mix substitution buffer were mixed with 2.0 mL overlay agar and poured on minimal agar plates.

3.7 Data Evaluation

3.7.1 Data Recording

The colonies were counted using a validated computer system (cf. 3.8, Major computerized systems), which was connected to a PC with printer to print out the individual values, the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). Due to reduced background growth of the bacteria the colonies were partly counted manually.

3.7.2 Acceptability of the Assay

The *Salmonella typhimurium* and *Escherichia coli* reverse mutation assay is considered acceptable if it meets the following criteria:

- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of our historical data
- the positive control substances should produce an increase above the threshold of twice (strains TA 98, TA 100, and WP2 *uvrA*) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control
- a minimum of five analysable dose levels should be present with at least three dose levels showing no signs of toxic effects, evident as a reduction in the number of revertants below the indication factor of 0.5.

3.7.3 Evaluation of Results

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA 98, TA 100, and WP2 *uvrA*) or thrice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed.

A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

An increase exceeding the threshold at only one concentration is judged as biologically relevant if reproduced in an independent second experiment.

A dose dependent increase in the number of revertant colonies below the threshold is regarded as an indication of a mutagenic potential if reproduced in an independent second experiment. However, whenever the colony counts remain within the historical range of negative and solvent controls such an increase is not considered biologically relevant.

3.7.4 Biometry

According to the OECD guideline 471, a statistical analysis of the data is not mandatory.

3.8 Major Computerized Systems

Petri Viewer Sorcerer Colony Counter 3.0 (Perceptive Instruments Ltd, Suffolk CB9 7BN, UK) with the software program Ames Study Manager (v1.24).

4 DEVIATIONS FROM STUDY PLAN

The following deviations from study plan occurred:

Due to the toxicity of the solvent ethanol the appropriate volume of test solution is **50µL** in the second experiment.

These deviations were considered to have not affected the integrity or validity of the study.

5 ARCHIVING

Records and documentation relating to this study will be maintained in the archives of Envigo CRS GmbH for a period of 4 years from the date on which the Study Director signs the final report. This will include but may not be limited to the Study Plan, raw data, Report and test item generated during the course of this study.

At termination of the aforementioned period, the records and documentation will be transferred to the GLP compliant Archive of Envigo CRS (Switzerland) Ltd. at Füllinsdorf, Switzerland, for further archiving up to a total archiving period of 15 years.

Samples and specimens that no longer afford evaluation will be discarded in accordance with Standard Operating Procedures and without further notice.

Envigo will retain in its archive the study plan and final report, and any amendments indefinitely.

6 RESULTS AND DISCUSSION

The test item Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-,(3aR,4S,5R,7S,7aR)-rel- was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I and Ia) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and the *Escherichia coli* strain WP2 *uvrA*. Due to a calculation error in experiment I the applied concentrations were not in compliance with the OECD 471 guideline. Therefore the experiment was repeated and reported as experiment Ia. Since the repeat was performed after experiment II and the dose selection for experiment II was based on the results of experiment I all collected data are reported.
The assay was performed in three independent experiments all with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at the following concentrations:

Pre-Experiment/Experiment I: 2.7; 9.0; 29.6; 89.6; 298.2; 895.6; 2239; and 4478 µg/plate

Pre-Experiment/Experiment Ia: 3; 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment II:

TA 100: 3; 10; 33; 100; 333; 1000; and 2500 µg/plate
All remaining strains: 10; 33; 100; 333; 1000; 2500; and 5000 µg/plate

The test item precipitated in the overlay agar in the test tubes at a concentration of 895.6 µg/plate and above in experiment I; from 1000 to 5000 µg/plate in the experiment Ia and at 2500 and 5000 µg/plate in experiment II. No precipitation of the test item in the overlay agar on the incubated agar plates was observed.

The plates incubated with the test item showed reduced background growth at the following concentrations (µg/plate):

Strain	Experiment I	
	without S9 mix	with S9 mix
TA 1535	895.6 – 4478	895.6 – 4478
TA 1537	895.6 – 4478	895.6 – 4478
TA 98	895.6 – 4478	895.6 – 4478
TA 100	895.6 – 4478	895.6 – 4478
WP2 uvrA	895.6 – 4478	2239 – 4478

Strain	Experiment Ia		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	2500 – 5000	1000 – 5000	2500 – 5000	2500 – 5000
TA 1537	2500 – 5000	1000 – 5000	2500 – 5000	1000 – 5000
TA 98	5000	1000 – 5000	2500 – 5000	1000 – 5000
TA 100	5000	2500 – 5000	1000 – 2500	333 – 2500
WP2 uvrA	5000	5000	5000	2500 – 5000

Toxic effects, evident as a reduction in the number of revertants (below the induction factor of 0.5), were observed at the following concentrations (µg/plate):

Strain	Experiment I	
	without S9 mix	with S9 mix
TA 1535	/	/
TA 1537	4478	/
TA 98	4478	4478
TA 100	298.2 – 4478	298.2 – 4478
WP2 uvrA	4478	/

/ = no toxic effect (induction factor > 0.5)

Strain	Experiment Ia		Experiment II	
	without S9 mix	with S9 mix	without S9 mix	with S9 mix
TA 1535	5000	/	/	5000
TA 1537	5000	5000	2500 – 5000	2500 – 5000
TA 98	/	2500 – 5000	2500 – 5000	1000 – 5000
TA 100	1000 – 5000	333 – 5000	100 – 2500	100 – 2500
WP2 uvrA	/	/	/	/

/ = no toxic effect (induction factor > 0.5)

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel- at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in induced revertant colonies. In experiment Ia in the absence of metabolic activation, the data in the solvent control of strain TA 98 were slightly above our historical control range. Since this deviation is rather small, this effect is considered to be based upon biologically irrelevant fluctuations in the number of colonies.

7 CONCLUSION

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test item did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

8 REFERENCES

Ames, B.N., J. McCann, and E. Yamasaki (1977)
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
In: B.J. Kilbey et al. (Eds.)"Handbook of Mutagenicity Test Procedures" Elsevier, Amsterdam, 1-17

de Serres F.J. and M.D. Shelby (1979)
Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
Mutation Res. 64, 159-165

Hollstein,M., J. McCann, F.A. Angelosanto, and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226

Green, M.H.L. and W.J. Muriel (1976)
Mutagen Testing Using TRP^{+} Reversion in Escherichia Coli
Mutation. Res. 38, 3- 32

Maron D.M., J. Katzenellenbogen, and B.N. Ames (1981)
Compatibility of organic solvents with the Salmonella/Microsome Test
Mutation Res. 88, 343-350

Maron D.M., Ames, B.N. (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215

TABLES

Table 1 Summary of Experiment I

Study Name: 1783503
Experiment: 1783503_VV_Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 13.07.2016
Date Counted: 19.07.2016

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Ethanol		12 ± 3	12 ± 3	28 ± 13	161 ± 2	55 ± 8
	Untreated		13 ± 3	10 ± 1	31 ± 7	197 ± 10	56 ± 12
	Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	2.7 µg	12 ± 2	9 ± 2	26 ± 2	150 ± 4	44 ± 11
		9.0 µg	14 ± 2	7 ± 2	26 ± 1	156 ± 6	48 ± 5
		29.6 µg	11 ± 4	9 ± 3	27 ± 1	156 ± 11	59 ± 10
		89.6 µg	9 ± 3	9 ± 2	29 ± 5	100 ± 6	45 ± 8
		298.2 µg	11 ± 3	7 ± 3	22 ± 6	68 ± 7	40 ± 0
		895.6 µg	8 ± 3 R	8 ± 2 R	30 ± 4 R	58 ± 5 R	32 ± 5 R
		2239 µg	8 ± 2 R	6 ± 1 M R	22 ± 5 R	51 ± 6 R	36 ± 1 R
		4478 µg	10 ± 3 M R	2 ± 1 M R	12 ± 2 M R	13 ± 3 M R	24 ± 4 R M
	NaN3	10 µg	997 ± 40			2043 ± 209	
	4-NOPD	10 µg			429 ± 5		
	4-NOPD	50 µg		73 ± 13			
	MMS	2.0 µL					950 ± 56
With Activation	Ethanol		20 ± 1	11 ± 1	47 ± 6	152 ± 17	53 ± 7
	Untreated		13 ± 2	13 ± 3	43 ± 8	147 ± 9	56 ± 5
	Mixture of 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4R,5S,7R,7aR)-rel- and 4,7-Methano-1H-indene, 5-ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	2.7 µg	19 ± 4	14 ± 5	41 ± 3	127 ± 16	53 ± 7
		9.0 µg	17 ± 6	14 ± 2	50 ± 8	146 ± 9	55 ± 8
		29.6 µg	14 ± 2	16 ± 4	51 ± 5	147 ± 8	56 ± 4
		89.6 µg	12 ± 4	15 ± 5	38 ± 4	92 ± 8	58 ± 5
		298.2 µg	11 ± 1	15 ± 4	43 ± 7	52 ± 7	46 ± 3
		895.6 µg	12 ± 2 R	14 ± 3 M R	45 ± 9 R	22 ± 7 M R	44 ± 6
		2239 µg	16 ± 3 M R	14 ± 5 M R	28 ± 8 M R	4 ± 1 M R	41 ± 9 R
		4478 µg	15 ± 5 M R	9 ± 3 M R	15 ± 5 M R	2 ± 1 M R	24 ± 3 M R
	2-AA	2.5 µg	339 ± 29	313 ± 13	4136 ± 548	3564 ± 163	
	2-AA	10.0 µg					385 ± 21

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Table 2 Summary of Experiment Ia

Study Name: 1783503 Study Code: Envigo 1783503
Experiment: 1783503 VVa Plate Date Plated: 21.09.2016
Assay Conditions: Date Counted: 28.09.2016

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Ethanol		13 ± 3	15 ± 6	40 ± 8	185 ± 8	45 ± 4
	Untreated		14 ± 4	19 ± 4	43 ± 8	193 ± 9	48 ± 10
	Mixture of 4,7-Methano-	3 µg	13 ± 5	14 ± 6	48 ± 6	189 ± 13	34 ± 10
	1H-indene, 5-	10 µg	14 ± 3	14 ± 6	42 ± 4	184 ± 23	43 ± 11
	ethoxyoctahydro-,	33 µg	15 ± 3	14 ± 2	45 ± 2	164 ± 4	45 ± 10
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	14 ± 3	14 ± 6	33 ± 3	98 ± 2	35 ± 6
	and 4,7-Methano-1H-	333 µg	11 ± 4	9 ± 2	35 ± 5	88 ± 2	34 ± 8
	indene, 5-ethoxyoctahydro-,	1000 µg	7 ± 2	14 ± 6	29 ± 6	79 ± 6	33 ± 4
	(3aR,4S,5R,7S,7aR)-rel-	2500 µg	8 ± 1 [R]	8 ± 3 [R]	28 ± 4	56 ± 10	32 ± 8
		5000 µg	4 ± 1 [M R]	5 ± 2 [M R]	31 ± 6 [R]	45 ± 13 [R]	29 ± 3 [R]
	NaN3	10 µg	1460 ± 78			2318 ± 273	
	4-NOPD	10 µg			451 ± 31		
	4-NOPD	50 µg		85 ± 3			
	MMS	2.0 µL					1087 ± 82
With Activation	Ethanol		14 ± 5	16 ± 4	43 ± 3	186 ± 7	46 ± 12
	Untreated		11 ± 6	20 ± 5	53 ± 7	171 ± 14	52 ± 14
	Mixture of 4,7-Methano-	3 µg	13 ± 6	16 ± 1	51 ± 7	175 ± 21	53 ± 14
	1H-indene, 5-	10 µg	15 ± 1	16 ± 6	45 ± 5	164 ± 10	50 ± 12
	ethoxyoctahydro-,	33 µg	16 ± 4	19 ± 5	50 ± 4	155 ± 13	58 ± 9
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	15 ± 5	15 ± 6	46 ± 1	105 ± 12	56 ± 17
	and 4,7-Methano-1H-	333 µg	13 ± 2	18 ± 6	41 ± 4	54 ± 6	57 ± 5
	indene, 5-ethoxyoctahydro-,	1000 µg	14 ± 2 [R]	17 ± 2 [R]	46 ± 5 [R]	53 ± 6	32 ± 2
	(3aR,4S,5R,7S,7aR)-rel-	2500 µg	9 ± 2 [M R]	15 ± 3 [M R]	16 ± 3 [M R]	22 ± 4 [M R]	33 ± 5
		5000 µg	8 ± 1 [M R]	5 ± 2 [M R]	16 ± 4 [M R]	1 ± 1 [M R]	37 ± 5 [R]
	2-AA	2.5 µg	407 ± 3	189 ± 18	3315 ± 368	4072 ± 160	
	2-AA	10.0 µg					372 ± 14

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Table 3 Summary of Experiment II

Study Name: 1783503 Study Code: Envigo 1783503
Experiment: 1783503 HV2 Pre Date Plated: 05.08.2016
Assay Conditions: Date Counted: 11.08.2016

Metabolic Activation	Test Group	Dose Level (per plate)	Revertant Colony Counts (Mean ±SD)				
			TA 1535	TA 1537	TA 98	TA 100	WP2 uvrA
Without Activation	Ethanol		11 ± 2	10 ± 1	37 ± 6	192 ± 14	46 ± 9
	Untreated		9 ± 3	15 ± 5	29 ± 4	198 ± 24	37 ± 10
	Mixture of 4,7-Methano-	3 µg				190 ± 20	
	1H-indene, 5-	10 µg	12 ± 3	9 ± 3	33 ± 2	200 ± 29	45 ± 3
	ethoxyoctahydro-,	33 µg	14 ± 6	9 ± 3	33 ± 9	157 ± 12	51 ± 7
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	11 ± 1	9 ± 4	29 ± 8	75 ± 9	41 ± 1
	and 4,7-Methano-1H-	333 µg	12 ± 5	8 ± 2	24 ± 5	72 ± 22	48 ± 8
	indene, 5-ethoxyoctahydro-,	1000 µg	11 ± 2	9 ± 0	29 ± 8	53 ± 12 R	41 ± 11
	(3aR,4S,5R,7S,7aR)-rel-	2500 µg	8 ± 3 R	2 ± 1 R M	14 ± 3 M R	29 ± 8 M R	32 ± 6
		5000 µg	8 ± 2 R	0 ± 1 R M	8 ± 2 R M		28 ± 5 R
	NaN3	10 µg	1330 ± 7			2073 ± 75	
	4-NOPD	10 µg			528 ± 28		
	4-NOPD	50 µg		80 ± 11			
	MMS	2.0 µL					763 ± 73
With Activation	Ethanol		17 ± 6	18 ± 2	52 ± 5	198 ± 24	54 ± 2
	Untreated		10 ± 3	16 ± 7	35 ± 3	211 ± 17	50 ± 9
	Mixture of 4,7-Methano-	3 µg				194 ± 8	
	1H-indene, 5-	10 µg	16 ± 7	15 ± 1	52 ± 6	189 ± 13	45 ± 4
	ethoxyoctahydro-,	33 µg	15 ± 3	17 ± 3	50 ± 3	210 ± 12	59 ± 9
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	13 ± 6	12 ± 5	55 ± 4	86 ± 16	50 ± 8
	and 4,7-Methano-1H-	333 µg	12 ± 6	15 ± 6	41 ± 6	51 ± 16 R	43 ± 4
	indene, 5-ethoxyoctahydro-,	1000 µg	14 ± 3	9 ± 2 R M	12 ± 2 M R	24 ± 6 M R	44 ± 6
	(3aR,4S,5R,7S,7aR)-rel-	2500 µg	14 ± 5 R	5 ± 1 R M	9 ± 2 R M	7 ± 2 R M	40 ± 8 R
		5000 µg	4 ± 1 R M	3 ± 1 R M	0 ± 0 R M		29 ± 5 R
	2-AA	2.5 µg	373 ± 17	257 ± 27	4225 ± 757	4704 ± 300	
	2-AA	10.0 µg					363 ± 34

Key to Positive Controls

NaN3	sodium azide
2-AA	2-aminoanthracene
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Table 4 Individual Results of Experiment I

Study Name: 1783503
Experiment: 1783503_VV_Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 13.07.2016
Date Counted: 19.07.2016

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Mixture of 4,7-Methano-	2.7 µg	*12.0*	*1.7*	*1.0*	11, 11, 14
	1H-indene, 5-	9.0 µg	*14.0*	*1.7*	*1.2*	12, 15, 15
	ethoxyoctahydro-,	29.6 µg	*11.3*	*4.0*	*0.9*	15, 7, 12
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*9.3*	*2.5*	*0.8*	12, 7, 9
	and 4,7-Meth-ano-1H-	298.2 µg	*11.0*	*2.6*	*0.9*	10, 14, 9
	indene, 5-	895.6 µg	*8.0*	*2.6*	*0.7*	5 R, 9 R, 10 R
	ethoxyoctahydro-,	2239 µg	*7.7*	*2.3*	*0.6*	9 R, 9 R, 5 R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*9.7*	*3.1*	*0.8*	7 M R, 9 M R, 13 M R
	Ethanol		*12.0*	*3.0*		9, 15, 12
	Untreated Control		*12.7*	*3.2*		15, 14, 9
TA 1537	Mixture of 4,7-Methano-	2.7 µg	*9.0*	*1.7*	*0.8*	10, 10, 7
	1H-indene, 5-	9.0 µg	*7.0*	*2.0*	*0.6*	5, 7, 9
	ethoxyoctahydro-,	29.6 µg	*9.0*	*3.0*	*0.8*	9, 12, 6
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*9.0*	*2.0*	*0.8*	7, 9, 11
	and 4,7-Meth-ano-1H-	298.2 µg	*7.3*	*2.5*	*0.6*	7, 10, 5
	indene, 5-	895.6 µg	*8.3*	*2.1*	*0.7*	9 R, 10 R, 6 R
	ethoxyoctahydro-,	2239 µg	*6.0*	*1.0*	*0.5*	6 M R, 5 M R, 7 M R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*2.3*	*0.6*	*0.2*	3 M R, 2 M R, 2 M R
	Ethanol		*12.0*	*2.6*		11, 15, 10
	Untreated Control		*9.7*	*0.6*		9, 10, 10
TA 98	Mixture of 4,7-Methano-	2.7 µg	*26.3*	*1.5*	*0.9*	25, 26, 28
	1H-indene, 5-	9.0 µg	*26.0*	*1.0*	*0.9*	26, 27, 25
	ethoxyoctahydro-,	29.6 µg	*27.3*	*0.6*	*1.0*	27, 28, 27
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*29.3*	*5.1*	*1.0*	28, 35, 25
	and 4,7-Meth-ano-1H-	298.2 µg	*22.3*	*5.5*	*0.8*	16, 25, 26
	indene, 5-	895.6 µg	*30.0*	*3.6*	*1.1*	26 R, 33 R, 31 R
	ethoxyoctahydro-,	2239 µg	*21.7*	*5.1*	*0.8*	16 R, 26 R, 23 R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*12.0*	*2.0*	*0.4*	10 M R, 12 M R, 14 M R
	Ethanol		*28.0*	*12.5*		16, 27, 41
	Untreated Control		*31.3*	*6.5*		25, 38, 31
TA 100	Mixture of 4,7-Methano-	2.7 µg	*150.3*	*3.8*	*0.9*	146, 153, 152
	1H-indene, 5-	9.0 µg	*156.3*	*6.0*	*1.0*	157, 150, 162
	ethoxyoctahydro-,	29.6 µg	*156.0*	*10.5*	*1.0*	155, 167, 146
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*99.7*	*5.5*	*0.6*	94, 105, 100
	and 4,7-Meth-ano-1H-	298.2 µg	*68.3*	*6.5*	*0.4*	62, 68, 75
	indene, 5-	895.6 µg	*58.0*	*5.3*	*0.4*	56 R, 64 R, 54 R
	ethoxyoctahydro-,	2239 µg	*51.0*	*6.0*	*0.3*	45 R, 57 R, 51 R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*12.7*	*3.1*	*0.1*	16 M R, 10 M R, 12 M R
	Ethanol		*160.7*	*1.5*		161, 162, 159
	Untreated Control		*197.3*	*9.7*		195, 208, 189

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Study Name: 1783503
Experiment: 1783503_VV_Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 13.07.2016
Date Counted: 19.07.2016

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	Mixture of 4,7-Methano-	2.7 µg	*44.3*	*11.2*	*0.8*	40, 36, 57
	1H-indene, 5-	9.0 µg	*47.7*	*5.1*	*0.9*	49, 42, 52
	ethoxyoctahydro-,	29.6 µg	*58.7*	*9.7*	*1.1*	67, 61, 48
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*44.7*	*8.3*	*0.8*	42, 54, 38
	and 4,7-Meth-ano-1H-	298.2 µg	*40.0*	*0.0*	*0.7*	40, 40, 40
	indene, 5-	895.6 µg	*31.7*	*5.1*	*0.6*	26 R, 33 R, 36 R
	ethoxyoctahydro-,	2239 µg	*35.7*	*0.6*	*0.7*	35 R, 36 R, 36 R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*24.0*	*4.4*	*0.4*	29 R M, 22 R M, 21 R M
	Ethanol		*54.7*	*8.0*		47, 63, 54
	Untreated Control		*55.7*	*11.8*		42, 63, 62
TA 1535	**NaN3**	10 µg	*996.7*	*39.6*	*83.1*	1035, 999, 956
TA 1537	**4-NOPD**	50 µg	*72.7*	*12.9*	*6.1*	82, 58, 78
TA 98	**4-NOPD**	10 µg	*428.7*	*5.0*	*15.3*	424, 434, 428
TA 100	**NaN3**	10 µg	*2042.7*	*209.3*	*12.7*	2258, 2030, 1840
WP2 uvrA	**MMS**	2.0 µL	*950.0*	*56.0*	*17.4*	952, 1005, 893

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Study Name: 1783503
Experiment: 1783503_VV_Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 13.07.2016
Date Counted: 19.07.2016

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Mixture of 4,7-Methano-	2.7 µg	*19.3*	*3.5*	*1.0*	16, 19, 23
	1H-indene, 5-	9.0 µg	*16.7*	*5.7*	*0.8*	23, 12, 15
	ethoxyoctahydro-,	29.6 µg	*14.0*	*1.7*	*0.7*	12, 15, 15
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*12.3*	*3.5*	*0.6*	16, 12, 9
	and 4,7-Meth-ano-1H-	298.2 µg	*10.7*	*0.6*	*0.5*	11, 11, 10
	indene, 5-	895.6 µg	*12.3*	*1.5*	*0.6*	12 R, 14 R, 11 R
	ethoxyoctahydro-,	2239 µg	*16.0*	*2.6*	*0.8*	19 M R, 14 M R, 15 M R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*15.3*	*5.0*	*0.8*	16 M R, 10 M R, 20 M R
	Ethanol		*20.3*	*1.2*		21, 19, 21
	Untreated Control		*13.3*	*2.1*		11, 14, 15
TA 1537	Mixture of 4,7-Methano-	2.7 µg	*14.0*	*5.3*	*1.2*	12, 20, 10
	1H-indene, 5-	9.0 µg	*14.0*	*1.7*	*1.2*	12, 15, 15
	ethoxyoctahydro-,	29.6 µg	*15.7*	*4.2*	*1.4*	11, 17, 19
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*15.0*	*4.6*	*1.3*	20, 11, 14
	and 4,7-Meth-ano-1H-	298.2 µg	*15.0*	*4.0*	*1.3*	19, 15, 11
	indene, 5-	895.6 µg	*13.7*	*3.1*	*1.2*	11 M R, 13 M R, 17 M R
	ethoxyoctahydro-,	2239 µg	*14.0*	*4.6*	*1.2*	9 M R, 18 M R, 15 M R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*9.0*	*3.5*	*0.8*	13 M R, 7 M R, 7 M R
	Ethanol		*11.3*	*1.2*		12, 12, 10
	Untreated Control		*13.3*	*2.9*		10, 15, 15
TA 98	Mixture of 4,7-Methano-	2.7 µg	*40.7*	*3.2*	*0.9*	42, 43, 37
	1H-indene, 5-	9.0 µg	*49.7*	*7.6*	*1.1*	43, 58, 48
	ethoxyoctahydro-,	29.6 µg	*50.7*	*4.7*	*1.1*	49, 47, 56
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*38.0*	*4.4*	*0.8*	43, 35, 36
	and 4,7-Meth-ano-1H-	298.2 µg	*42.7*	*7.4*	*0.9*	51, 40, 37
	indene, 5-	895.6 µg	*45.3*	*8.6*	*1.0*	47 R, 53 R, 36 R
	ethoxyoctahydro-,	2239 µg	*27.7*	*7.6*	*0.6*	36 M R, 21 M R, 26 M R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*14.7*	*4.5*	*0.3*	19 M R, 10 M R, 15 M R
	Ethanol		*47.0*	*5.6*		41, 48, 52
	Untreated Control		*43.3*	*7.6*		52, 40, 38
TA 100	Mixture of 4,7-Methano-	2.7 µg	*126.7*	*15.5*	*0.8*	111, 127, 142
	1H-indene, 5-	9.0 µg	*146.3*	*9.3*	*1.0*	142, 157, 140
	ethoxyoctahydro-,	29.6 µg	*147.0*	*7.8*	*1.0*	138, 151, 152
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*92.0*	*7.5*	*0.6*	91, 85, 100
	and 4,7-Meth-ano-1H-	298.2 µg	*52.0*	*7.0*	*0.3*	45, 52, 59
	indene, 5-	895.6 µg	*21.7*	*7.1*	*0.1*	28 M R, 23 M R, 14 M R
	ethoxyoctahydro-,	2239 µg	*4.0*	*1.0*	*0.0*	5 M R, 3 M R, 4 M R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*1.7*	*1.2*	*0.0*	3 M R, 1 M R, 1 M R
	Ethanol		*152.0*	*16.5*		153, 168, 135
	Untreated Control		*146.7*	*9.0*		141, 142, 157

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

Study Name: 1783503
Experiment: 1783503_VV_Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 13.07.2016
Date Counted: 19.07.2016

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	Mixture of 4,7-Methano-	2.7 µg	*52.7*	*6.5*	*1.0*	59, 53, 46
	1H-indene, 5-	9.0 µg	*55.0*	*7.9*	*1.0*	49, 64, 52
	ethoxyoctahydro-,	29.6 µg	*55.7*	*4.0*	*1.1*	58, 58, 51
	(3aR,4R,5S,7R,7aR)-rel-	89.6 µg	*58.0*	*4.6*	*1.1*	54, 57, 63
	and 4,7-Meth-ano-1H-	298.2 µg	*46.0*	*2.6*	*0.9*	47, 43, 48
	indene, 5-	895.6 µg	*44.0*	*6.1*	*0.8*	40, 51, 41
	ethoxyoctahydro-,	2239 µg	*41.3*	*9.0*	*0.8*	31 R, 46 R, 47 R
	(3aR,4S,5R,7S,7aR)-rel-	4478 µg	*24.0*	*3.0*	*0.5*	21 M R, 24 M R, 27 M R
	Ethanol		*52.7*	*6.7*		57, 45, 56
	Untreated Control		*56.3*	*4.5*		61, 52, 56
TA 1535	**2-AA**	2.5 µg	*339.3*	*29.1*	*16.7*	367, 342, 309
TA 1537	**2-AA**	2.5 µg	*313.3*	*13.4*	*27.6*	323, 319, 298
TA 98	**2-AA**	2.5 µg	*4135.7*	*547.7*	*88.0*	3533, 4271, 4603
TA 100	**2-AA**	2.5 µg	*3563.7*	*162.9*	*23.4*	3439, 3504, 3748
WP2 uvrA	**2-AA**	10.0 µg	*385.0*	*20.7*	*7.3*	404, 363, 388

Key to Positive Controls

2-AA	2-aminoanthracene

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

Table 5 Individual Results of Experiment Ia

Study Name: 1783503
Experiment: 1783503 VVa Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 21.09.2016
Date Counted: 28.09.2016

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Mixture of 4,7-Methano-	3 µg	*12.7*	*4.9*	*1.0*	16, 15, 7
	1H-indene, 5-	10 µg	*13.7*	*3.2*	*1.1*	10, 16, 15
	ethoxyoctahydro-,	33 µg	*15.0*	*3.5*	*1.2*	11, 17, 17
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*13.7*	*2.9*	*1.1*	12, 17, 12
	and 4,7-Meth-ano-1H-	333 µg	*11.0*	*3.6*	*0.9*	14, 12, 7
	indene, 5-	1000 µg	*7.3*	*1.5*	*0.6*	7, 9, 6
	ethoxyoctahydro-,	2500 µg	*7.7*	*1.2*	*0.6*	7 R, 7 R, 9 R
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*3.7*	*1.2*	*0.3*	3 M R, 3 M R, 5 M R
	Ethanol		*12.7*	*2.9*		11, 11, 16
	Untreated Control		*14.0*	*4.4*		9, 17, 16
TA 1537	Mixture of 4,7-Methano-	3 µg	*13.7*	*5.7*	*0.9*	12, 9, 20
	1H-indene, 5-	10 µg	*14.3*	*5.9*	*1.0*	21, 10, 12
	ethoxyoctahydro-,	33 µg	*13.7*	*1.5*	*0.9*	15, 14, 12
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*13.7*	*5.5*	*0.9*	20, 11, 10
	and 4,7-Meth-ano-1H-	333 µg	*9.0*	*2.0*	*0.6*	9, 11, 7
	indene, 5-	1000 µg	*13.7*	*6.4*	*0.9*	21, 9, 11
	ethoxyoctahydro-,	2500 µg	*8.0*	*3.5*	*0.5*	12 R, 6 R, 6 R
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*4.7*	*1.5*	*0.3*	3 M R, 5 M R, 6 M R
	Ethanol		*15.0*	*6.1*		12, 11, 22
	Untreated Control		*19.0*	*3.6*		22, 20, 15
TA 98	Mixture of 4,7-Methano-	3 µg	*48.3*	*5.5*	*1.2*	51, 52, 42
	1H-indene, 5-	10 µg	*42.0*	*4.0*	*1.1*	38, 42, 46
	ethoxyoctahydro-,	33 µg	*45.3*	*2.1*	*1.1*	43, 47, 46
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*32.7*	*2.9*	*0.8*	31, 36, 31
	and 4,7-Meth-ano-1H-	333 µg	*35.0*	*5.0*	*0.9*	35, 40, 30
	indene, 5-	1000 µg	*28.7*	*5.9*	*0.7*	31, 22, 33
	ethoxyoctahydro-,	2500 µg	*28.3*	*3.5*	*0.7*	25, 32, 28
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*30.7*	*6.0*	*0.8*	30 R, 25 R, 37 R
	Ethanol		*40.0*	*8.2*		38, 33, 49
	Untreated Control		*43.0*	*7.5*		51, 42, 36
TA 100	Mixture of 4,7-Methano-	3 µg	*189.3*	*13.4*	*1.0*	174, 199, 195
	1H-indene, 5-	10 µg	*184.3*	*22.7*	*1.0*	167, 176, 210
	ethoxyoctahydro-,	33 µg	*164.3*	*3.5*	*0.9*	164, 168, 161
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*98.3*	*2.1*	*0.5*	99, 96, 100
	and 4,7-Meth-ano-1H-	333 µg	*88.3*	*1.5*	*0.5*	87, 90, 88
	indene, 5-	1000 µg	*78.7*	*5.5*	*0.4*	79, 84, 73
	ethoxyoctahydro-,	2500 µg	*56.0*	*9.6*	*0.3*	49, 67, 52
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*45.3*	*12.9*	*0.2*	56 R, 31 R, 49 R
	Ethanol		*184.7*	*8.3*		182, 178, 194
	Untreated Control		*192.7*	*9.3*		203, 185, 190

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Study Name: 1783503
Experiment: 1783503 VVa Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 21.09.2016
Date Counted: 28.09.2016

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	Mixture of 4,7-Methano-	3 µg	*33.7*	*9.7*	*0.7*	23, 36, 42
	1H-indene, 5-	10 µg	*42.7*	*10.7*	*0.9*	31, 45, 52
	ethoxyoctahydro-,	33 µg	*45.0*	*10.4*	*1.0*	51, 51, 33
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*35.3*	*5.5*	*0.8*	41, 30, 35
	and 4,7-Meth-ano-1H-	333 µg	*34.3*	*7.6*	*0.8*	36, 26, 41
	indene, 5-	1000 µg	*33.3*	*4.2*	*0.7*	38, 32, 30
	ethoxyoctahydro-,	2500 µg	*31.7*	*8.4*	*0.7*	37, 22, 36
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*28.7*	*3.1*	*0.6*	32 R, 26 R, 28 R
	Ethanol		*45.3*	*3.5*		42, 45, 49
	Untreated Control		*48.0*	*9.5*		59, 43, 42
TA 1535	**NaN3**	10 µg	*1459.7*	*78.1*	*115.2*	1496, 1370, 1513
TA 1537	**4-NOPD**	50 µg	*84.7*	*2.5*	*5.6*	82, 85, 87
TA 98	**4-NOPD**	10 µg	*451.3*	*31.2*	*11.3*	417, 459, 478
TA 100	**NaN3**	10 µg	*2317.7*	*272.7*	*12.6*	2366, 2024, 2563
WP2 uvrA	**MMS**	2.0 µL	*1087.3*	*82.1*	*24.0*	1152, 1115, 995

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

R	Reduced background growth

Study Name: 1783503
Experiment: 1783503 VVa Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 21.09.2016
Date Counted: 28.09.2016

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Mixture of 4,7-Methano-	3 µg	*13.0*	*6.1*	*0.9*	9, 10, 20
	1H-indene, 5-	10 µg	*14.7*	*0.6*	*1.0*	15, 14, 15
	ethoxyoctahydro-,	33 µg	*15.7*	*4.2*	*1.1*	19, 17, 11
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*15.0*	*4.6*	*1.0*	11, 20, 14
	and 4,7-Meth-ano-1H-	333 µg	*13.3*	*2.1*	*0.9*	14, 11, 15
	indene, 5-	1000 µg	*14.3*	*2.1*	*1.0*	12 R, 15 R, 16 R
	ethoxyoctahydro-,	2500 µg	*8.7*	*1.5*	*0.6*	10 M R, 7 M R, 9 M R
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*8.3*	*0.6*	*0.6*	8 M R, 8 M R, 9 M R
	Ethanol		*14.3*	*4.5*		19, 14, 10
	Untreated Control		*11.3*	*5.5*		6, 11, 17
TA 1537	Mixture of 4,7-Methano-	3 µg	*16.0*	*1.0*	*1.0*	15, 17, 16
	1H-indene, 5-	10 µg	*15.7*	*5.7*	*1.0*	14, 11, 22
	ethoxyoctahydro-,	33 µg	*19.0*	*4.6*	*1.2*	14, 23, 20
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*15.0*	*6.0*	*0.9*	15, 21, 9
	and 4,7-Meth-ano-1H-	333 µg	*18.0*	*6.1*	*1.1*	15, 14, 25
	indene, 5-	1000 µg	*17.3*	*2.3*	*1.1*	16 R, 16 R, 20 R
	ethoxyoctahydro-,	2500 µg	*15.3*	*3.1*	*0.9*	12 M R, 16 M R, 18 M R
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*4.7*	*2.1*	*0.3*	7 M R, 3 M R, 4 M R
	Ethanol		*16.3*	*4.0*		14, 21, 14
	Untreated Control		*20.0*	*4.6*		25, 19, 16
TA 98	Mixture of 4,7-Methano-	3 µg	*51.3*	*6.8*	*1.2*	59, 49, 46
	1H-indene, 5-	10 µg	*45.0*	*4.6*	*1.0*	49, 46, 40
	ethoxyoctahydro-,	33 µg	*50.0*	*3.6*	*1.2*	51, 53, 46
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*46.0*	*1.0*	*1.1*	46, 45, 47
	and 4,7-Meth-ano-1H-	333 µg	*41.3*	*3.5*	*1.0*	45, 41, 38
	indene, 5-	1000 µg	*46.0*	*5.2*	*1.1*	52 R, 43 R, 43 R
	ethoxyoctahydro-,	2500 µg	*16.0*	*2.6*	*0.4*	15 M R, 14 M R, 19 M R
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*16.0*	*4.4*	*0.4*	21 M R, 14 M R, 13 M R
	Ethanol		*43.0*	*3.0*		46, 40, 43
	Untreated Control		*53.3*	*7.2*		57, 45, 58
TA 100	Mixture of 4,7-Methano-	3 µg	*175.0*	*20.8*	*0.9*	151, 187, 187
	1H-indene, 5-	10 µg	*163.7*	*9.7*	*0.9*	166, 153, 172
	ethoxyoctahydro-,	33 µg	*155.0*	*13.1*	*0.8*	167, 157, 141
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*104.7*	*12.1*	*0.6*	109, 114, 91
	and 4,7-Meth-ano-1H-	333 µg	*54.3*	*6.4*	*0.3*	59, 57, 47
	indene, 5-	1000 µg	*53.3*	*6.4*	*0.3*	46, 57, 57
	ethoxyoctahydro-,	2500 µg	*22.3*	*4.0*	*0.1*	23 M R, 18 M R, 26 M R
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*0.7*	*0.6*	*0.0*	1 M R, 0 M R, 1 M R
	Ethanol		*186.0*	*6.6*		179, 187, 192
	Untreated Control		*171.3*	*14.4*		182, 155, 177

Key to Plate Postfix Codes

M	Manual count
R	Reduced background growth

Study Name: 1783503
Experiment: 1783503 VVa Plate
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 21.09.2016
Date Counted: 28.09.2016

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
WP2 uvrA	Mixture of 4,7-Methano-	3 µg	*52.7*	*14.4*	*1.2*	61, 61, 36
	1H-indene, 5-	10 µg	*50.0*	*12.2*	*1.1*	56, 36, 58
	ethoxyoctahydro-,	33 µg	*57.7*	*9.1*	*1.3*	68, 51, 54
	(3aR,4R,5S,7R,7aR)-rel-	100 µg	*56.0*	*17.4*	*1.2*	68, 36, 64
	and 4,7-Meth-ano-1H-	333 µg	*56.7*	*5.1*	*1.2*	58, 61, 51
	indene, 5-	1000 µg	*31.7*	*1.5*	*0.7*	33, 30, 32
	ethoxyoctahydro-,	2500 µg	*33.0*	*5.3*	*0.7*	35, 37, 27
	(3aR,4S,5R,7S,7aR)-rel-	5000 µg	*37.3*	*5.0*	*0.8*	42 R, 32 R, 38 R
	Ethanol		*45.7*	*11.7*		41, 59, 37
	Untreated Control		*52.0*	*14.0*		68, 42, 46
TA 1535	**2-AA**	2.5 µg	*407.0*	*3.5*	*28.4*	409, 403, 409
TA 1537	**2-AA**	2.5 µg	*189.0*	*18.0*	*11.6*	174, 184, 209
TA 98	**2-AA**	2.5 µg	*3315.0*	*368.4*	*77.1*	3002, 3222, 3721
TA 100	**2-AA**	2.5 µg	*4072.3*	*159.5*	*21.9*	4254, 3955, 4008
WP2 uvrA	**2-AA**	10.0 µg	*372.3*	*13.8*	*8.2*	362, 388, 367

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

R Reduced background growth

Table 6 Individual Results of Experiment II

Study Name: 1783503
Experiment: 1783503 HV2 Pre
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 05.08.2016
Date Counted: 11.08.2016

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*11.7*	*2.9*	*1.1*	15, 10, 10
		33 µg	*14.0*	*6.2*	*1.3*	16, 19, 7
		100 µg	*10.7*	*0.6*	*1.0*	11, 11, 10
		333 µg	*11.7*	*4.5*	*1.1*	12, 16, 7
		1000 µg	*11.3*	*2.3*	*1.1*	14, 10, 10
		2500 µg	*8.3*	*3.2*	*0.8*	7 R, 12 R, 6 R
		5000 µg	*8.0*	*1.7*	*0.8*	10 R, 7 R, 7 R
	Ethanol		*10.7*	*1.5*		12, 9, 11
	Untreated Control		*9.3*	*3.1*		10, 6, 12
TA 1537	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*9.0*	*3.0*	*0.9*	6, 9, 12
		33 µg	*9.3*	*2.5*	*1.0*	7, 9, 12
		100 µg	*9.0*	*3.6*	*0.9*	12, 5, 10
		333 µg	*8.3*	*2.3*	*0.9*	11, 7, 7
		1000 µg	*9.0*	*0.0*	*0.9*	9, 9, 9
		2500 µg	*2.3*	*0.6*	*0.2*	3 R M, 2 R M, 2 R M
		5000 µg	*0.3*	*0.6*	*0.0*	1 R M, 0 R M, 0 R M
	Ethanol		*9.7*	*0.6*		9, 10, 10
	Untreated Control		*15.0*	*5.3*		17, 19, 9
TA 98	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*33.3*	*1.5*	*0.9*	32, 35, 33
		33 µg	*32.7*	*8.7*	*0.9*	40, 23, 35
		100 µg	*29.3*	*7.8*	*0.8*	27, 23, 38
		333 µg	*24.3*	*4.7*	*0.7*	28, 19, 26
		1000 µg	*29.3*	*7.8*	*0.8*	27, 38, 23
		2500 µg	*14.3*	*3.2*	*0.4*	18 M R, 13 M R, 12 M R
		5000 µg	*7.7*	*1.5*	*0.2*	6 R M, 9 R M, 8 R M
	Ethanol		*37.0*	*5.6*		36, 32, 43
	Untreated Control		*28.7*	*3.8*		26, 33, 27
TA 100	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	3 µg	*190.3*	*19.7*	*1.0*	181, 213, 177
		10 µg	*199.7*	*28.5*	*1.0*	172, 229, 198
		33 µg	*157.0*	*11.5*	*0.8*	145, 168, 158
		100 µg	*75.3*	*8.6*	*0.4*	77, 83, 66
		333 µg	*72.0*	*21.6*	*0.4*	48, 78, 90
		1000 µg	*53.3*	*11.7*	*0.3*	62 R, 40 R, 58 R
		2500 µg	*29.3*	*8.1*	*0.2*	22 M R, 38 M R, 28 M R
	Ethanol		*192.3*	*13.6*		185, 208, 184
	Untreated Control		*197.7*	*23.7*		225, 183, 185
WP2 uvrA	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*44.7*	*3.2*	*1.0*	47, 41, 46
		33 µg	*51.0*	*7.0*	*1.1*	54, 43, 56
		100 µg	*41.0*	*1.0*	*0.9*	40, 41, 42
		333 µg	*48.0*	*7.9*	*1.1*	45, 57, 42
		1000 µg	*40.7*	*11.0*	*0.9*	30, 52, 40
		2500 µg	*32.3*	*6.4*	*0.7*	25, 37, 35
		5000 µg	*28.0*	*5.0*	*0.6*	23 R, 28 R, 33 R
	Ethanol		*45.7*	*8.5*		46, 54, 37
	Untreated Control		*37.0*	*10.0*		27, 37, 47

Key to Plate Postfix Codes

R Reduced background growth
M Manual count

Study Name: 1783503
Experiment: 1783503 HV2 Pre
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 05.08.2016
Date Counted: 11.08.2016

Without metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**NaN3**	10 µg	*1329.7*	*6.8*	*124.7*	1332, 1322, 1335
TA 1537	**4-NOPD**	50 µg	*80.3*	*11.0*	*8.3*	93, 74, 74
TA 98	**4-NOPD**	10 µg	*528.0*	*28.2*	*14.3*	532, 554, 498
TA 100	**NaN3**	10 µg	*2073.3*	*75.4*	*10.8*	2037, 2023, 2160
WP2 uvrA	**MMS**	2.0 µL	*762.7*	*73.1*	*16.7*	718, 847, 723

Key to Positive Controls

NaN3	sodium azide
4-NOPD	4-nitro-o-phenylene-diamine
MMS	methyl methane sulfonate

Key to Plate Postfix Codes

Study Name: 1783503
Experiment: 1783503 HV2 Pre
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 05.08.2016
Date Counted: 11.08.2016

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*16.0*	*6.6*	*0.9*	22, 17, 9
		33 µg	*15.0*	*2.6*	*0.9*	12, 17, 16
		100 µg	*13.3*	*6.0*	*0.8*	7, 19, 14
		333 µg	*11.7*	*6.4*	*0.7*	19, 7, 9
		1000 µg	*13.7*	*3.2*	*0.8*	16, 15, 10
		2500 µg	*13.7*	*4.7*	*0.8*	12 R, 19 R, 10 R
		5000 µg	*4.0*	*1.0*	*0.2*	5 R M, 3 R M, 4 R M
	Ethanol		*17.3*	*5.5*		17, 23, 12
	Untreated Control		*10.3*	*2.9*		12, 12, 7
TA 1537	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*14.7*	*0.6*	*0.8*	15, 15, 14
		33 µg	*16.7*	*3.1*	*0.9*	16, 20, 14
		100 µg	*11.7*	*4.5*	*0.6*	7, 12, 16
		333 µg	*15.0*	*5.6*	*0.8*	10, 21, 14
		1000 µg	*9.0*	*2.0*	*0.5*	11 R M, 7 R M, 9 R M
		2500 µg	*5.0*	*1.0*	*0.3*	6 R M, 5 R M, 4 R M
		5000 µg	*3.0*	*1.0*	*0.2*	4 R M, 2 R M, 3 R M
	Ethanol		*18.0*	*1.7*		19, 19, 16
	Untreated Control		*15.7*	*6.7*		23, 10, 14
TA 98	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*51.7*	*5.5*	*1.0*	48, 58, 49
		33 µg	*50.3*	*3.1*	*1.0*	51, 47, 53
		100 µg	*54.7*	*3.8*	*1.1*	53, 59, 52
		333 µg	*41.3*	*5.7*	*0.8*	43, 35, 46
		1000 µg	*12.0*	*2.0*	*0.2*	14 M R, 12 M R, 10 M R
		2500 µg	*8.7*	*2.1*	*0.2*	8 R M, 11 R M, 7 R M
		5000 µg	*0.0*	*0.0*	*0.0*	0 R M, 0 R M, 0 R M
	Ethanol		*52.0*	*4.6*		57, 48, 51
	Untreated Control		*35.3*	*3.1*		38, 32, 36
TA 100	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	3 µg	*193.7*	*7.6*	*1.0*	199, 185, 197
		10 µg	*189.3*	*12.7*	*1.0*	187, 178, 203
		33 µg	*209.7*	*11.6*	*1.1*	223, 202, 204
		100 µg	*85.7*	*15.6*	*0.4*	69, 88, 100
		333 µg	*50.7*	*16.2*	*0.3*	36 R, 68 R, 48 R
		1000 µg	*23.7*	*6.4*	*0.1*	19 M R, 31 M R, 21 M R
		2500 µg	*6.7*	*2.1*	*0.0*	5 R M, 6 R M, 9 R M
	Ethanol		*197.7*	*24.2*		225, 179, 189
	Untreated Control		*211.3*	*17.1*		231, 200, 203
WP2 uvrA	Mixture of 4,7-Methano-1H -indene, 5-ethoxyocta-hydro-,(3aR,4R,5S,7R,7aR)-rel- and4,7-Methano-1H-indene, 5- ethoxyoctahydro-, (3aR,4S,5R,7S,7aR)-rel-	10 µg	*45.0*	*4.4*	*0.8*	40, 47, 48
		33 µg	*59.0*	*9.2*	*1.1*	51, 69, 57
		100 µg	*50.0*	*7.9*	*0.9*	53, 56, 41
		333 µg	*43.0*	*4.4*	*0.8*	46, 38, 45
		1000 µg	*44.0*	*6.1*	*0.8*	48, 37, 47
		2500 µg	*39.7*	*8.1*	*0.7*	35 R, 35 R, 49 R
		5000 µg	*29.0*	*5.3*	*0.5*	27 R, 25 R, 35 R
	Ethanol		*53.7*	*2.1*		52, 56, 53
	Untreated Control		*49.7*	*8.5*		56, 40, 53

Key to Plate Postfix Codes

R	Reduced background growth
M	Manual count

Study Name: 1783503
Experiment: 1783503 HV2 Pre
Assay Conditions:

Study Code: Envigo 1783503
Date Plated: 05.08.2016
Date Counted: 11.08.2016

With metabolic activation

Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA 1535	**2-AA**	2.5 µg	*373.3*	*17.5*	*21.5*	388, 378, 354
TA 1537	**2-AA**	2.5 µg	*256.7*	*27.2*	*14.3*	278, 226, 266
TA 98	**2-AA**	2.5 µg	*4224.7*	*756.9*	*81.2*	4095, 3541, 5038
TA 100	**2-AA**	2.5 µg	*4703.7*	*300.0*	*23.8*	4406, 4699, 5006
WP2 uvrA	**2-AA**	10.0 µg	*362.7*	*33.5*	*6.8*	363, 329, 396

Key to Positive Controls

2-AA 2-aminoanthracene

Key to Plate Postfix Codes

ANNEXES

Annex 1 Historical Data

These data represent the laboratory´s historical control data from January 2015 until December 2015 representing approx. 450 experiments (WP2 *uvrA* the historical data are based on approx. 200 experiments).

Strain		without S9 mix				with S9 mix			
		Mean	SD	Min	Max	Mean	SD	Min	Max
TA 1535	Solvent control	11	2.15	7	23	12	2.14	7	21
	Untreated control	12	2.97	6	24	12	2.71	7	26
	Positive control	1090	123.80	334	1372	392	62.85	176	549
TA1537	Solvent control	10	1.83	6	18	13	3.27	7	27
	Untreated control	10	2.29	6	20	14	3.72	7	25
	Positive control	83	12.28	55	131	175	44.44	82	327
TA 98	Solvent control	24	3.75	16	36	33	5.55	18	51
	Untreated control	26	4.72	15	43	36	5.83	17	56
	Positive control	344	51.13	211	599	3822	857.83	319	5048
TA 100	Solvent control	155	24.19	84	194	145	31.81	81	204
	Untreated control	174	21.92	90	206	170	23.62	93	212
	Positive control	1956	279.93	658	2528	3606	676.07	722	4940
WP2uvrA	Solvent control	41	5.72	27	63	51	6.91	37	72
	Untreated control	42	6.01	31	63	53	7.05	38	88
	Positive control	732	161.66	322	1066	362	72.26	212	858

Mean = mean value of revertants/plate
SD = standard deviation Min = minimal value/Max = maximal value

Annex 2 Certificate of Analysis

Annex 3 GLP Certificate

HESSEN

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance
(gemäß/according to § 19b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 2004/9/EG wurde durchgeführt in

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 2004/9/EEC at:

☒ Prüfeinrichtung/Test facility ☐ Prüfstandort/Test site

ENVIGO CRS GmbH
In den Leppsteinswiesen 19
64380 Roßdorf

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and adress)

Prüfungen nach Kategorien/Areas of Expertise
(gemäß/according chemVwV-GLP Nr. 5.3/OECD guidance)

2 Prüfungen zur Bestimmung der toxikologischen Eigenschaften	**2** Toxicity studies
3 Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)	**3** Mutagenicity studies
8 Analytische Prüfungen an biologischen Materialien	**8** Analytical studies on biological materials

13. – 16. Juli 2015
Datum der Inspektion/Date of Inspection
(Tag Monat Jahr/day month year)

Die genannte Prüfeinrichtung befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung die oben genannten Prüfungen unter Einhaltung der GLP- Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Im Auftrag

Th. Zimmermann, Referatsleiter, Wiesbaden, den **14. September 2015**
(Name und Funktion der verantwortlichen Person/
Name and function of responsible person)

HESSISCHES MINISTERIUM FÜR UMWELT, KLIMASCHUTZ, LANDWIRTSCHAFT UND VERBRAUCHERSCHUTZ

Hess. Ministerium für Umwelt, Klimaschutz, Landwirtschaft und Verbraucherschutz,
Mainzer Straße 80 D65189 Wiesbaden
(Name und Adresse der GLP-Überwachungsbehörde/Name and address of the GLP Monitoring Authority